

AMENDMENT

(Amendment under Article 11)

To Examiner of the Patent Office

1. Identification of International Application

PCT/JP03/03944

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#### 4. Subject of Amendment

##### Specification and Claims

#### 5. Contents of Amendment

(1) "DRUGS AND HEALTH FOODS INCLUDING ANTICANCER AGENTS AND IMMUNOPOTENTIATORS" described on page 1, lines 2-3 of the specification is amended to "DRUGS AND HEALTH FOODS INCLUDING IMMUNOPOTENTIATORS", and "... drugs and health foods including anticancer agents and immunopotentiators for inhibiting and preventing cancer and ... " described on the same page, lines 6 to 8, 14 to 15, and 23 to 25 are amended to "...drugs and health foods including immunopotentiators ..."and "... an anticancer agent or an immunopotentiator and a health food, ..." described on page 2, line 7-8 of the specification is amended to "... an immunopotentiator and a health food, ...".

(2) "... health food containing apple vinegar as a supplementary ingredient and an immune system strengthening food containing apple (*Malus domestica*) have been proposed. However, studies on the anticancer action or the immunopotentiating action of the apple fruit have not been conducted, except for the research by Miura et al., School of Medicine at Hirosaki University, which reports the finding that an apple polyphenol has an antitumor effect." described on page 4, lines 6 to 14 is amended to "... health

food containing apple vinegar as a supplementary ingredient have been proposed. However, studies on the immunopotentiating action of the apple fruit have not been conducted."

(3) "... and further studies ... as an anticancer agent or an immunopotentiator, ..." described on page 4, lines 18 to 19 is amended to "... and further studies ... as an immunoptentiator, ...".

(4) "An object of the invention described in claims 1 to 3 and 6 in the present inventions is to enhance the innate immune system such as natural killer cell, a macrophage or the like, or the activity of lymphocyte, and thus prevent or suppress cancer in addition to inhibiting the growth of cancer cells by using a fruit of the genus Malus such as apple (*Malus domestica*) and crabapple (*Malus pumila*) or a fruit of the genus Pyrus." described on page 5, lines 11 to 18 is amended to "An object of the invention described in claims 1 to 3 and 6 in the present inventions is to enhance the innate immune system such as natural killer cell, a macrophage or the like, or the activity of lymphocyte, and thus obtain a higher immunopotentiating effect by using both a mature fruit and an immature fruit or only a immature fruit of a fruit of the genus Malus such as apple (*Malus domestica*) and crabapple (*Malus pumila*) or a fruit of the genus Pyrus."

(5) "An object of the invention described in claims 5 and 7 is to obtain the higher antitumor effect or an immunopotentiating effect, in addition to the object of the invention described in claims 1 to 4 or 6" described on page 5, line 23 and page 6, line 1 is deleted.

(6) "... a substance derived therefrom, has an anticancer effect or a cancer-suppressing effect ..." on page 6, lines 5 to 7 is amended to "... a substance derived therefrom, has the higher immunopotentiating effect ...".

(7) "... is directed to a drug such as an anticancer agent or an immunopotentiator, which is characterized by comprising a fruit of the genus Malus ... a fruit of the genus Pyrus, or a substance derived therefrom as an active ingredient." on page 6, lines 11 to 16 is amended to "...is directed to a drug such as an immunopotentiator, which is characterized by comprising both a mature fruit and an immature fruit, or only an immature fruit of a fruit of the genus Malus ... a fruit of the genus Pyrus, or a substance derived therefrom as an active ingredient."

(8) "... an aqueous extract of the fruit of the genus Malus ..." described on page 6, lines 18 - 19 is amended to "... an aqueous extract of both the mature fruit and the immature fruit, or the immature fruit of the fruit of the genus Malus ...", "... the fruit of the genus Malus ... the fruit of the genus Pyrus ... alcohol ..." described on the same page, line 24 to

page 7, line 2 is amended to "... both the mature fruit and the immature fruit, or the immature fruit of the fruit of the genus Malus ... the fruit of the genus Pyrus ... alcohol ...", and "... the fruit of the genus Malus ... the fruit of the genus Pyrus, a substance therefrom ..." described on the same page, lines 10 - 12 is amended to "... both the mature fruit and the immature fruit, or only immature fruit of the fruit of the genus Malus ... the fruit of the genus Pyrus, a substance therefrom ...".

(9) "... when taken regularly, has the effect of preventing the onset of cancer, ..." described on page 7, lines 18 to 19 is amended to "... when taken regularly, obtain higher immunopotentiating effect and thus has the effect of preventing the onset of cancer, ... " and "an extract of the fruit of the genus Malus such as apple (Malus domestica)..." described on page 7, lines 22 - 25 is amended to "an extract of both the mature fruit and the immature fruit, or only immature fruit of the fruit of the genus Malus such as apple (Malus domestica)...".

(10) "The invention described in claim 5 ... similar action of preventing the onset of cancer." described on page 8, lines 10 to 22 is deleted.

(11) "... health food for inhibiting and preventing cancer or for enhancing immune system, comprising a fruit of the genus Malus such as apple (Malus domestica) and

crabapple (malus pumila), a fruit of the genus Pyrus, ..." described on page 8, line 23 to page 9, line 2 is amended to "... health food for enhancing immune system, comprising the mature fruit and the immature fruit, or only immature fruit of a fruit of the genus Malus such as apple (Malus domestica) and crabapple (Malus pumila), a fruit of the genus Pyrus, ..."

(12) "The invention described in claim 7 ... action of inhibiting the onset of cancer." described on page 9, line 15 to page 10, line 1 is deleted.

(13) "... crabapple (Malus domestica) ..." described on page 27, line 6 and the same page, line 20 is amended to "... crabapple (Malus pumila) ...".

(14) "Industrial applicability" described on page 30, line 25 is deleted, and "... a fruit of the genus Malus ... a fruit of the genus Pyrus or a substance derived therefrom ..." described on page 31, lines 3 to 6 is amended to "... both a mature fruit and an immature fruit, or only an immature fruit of a fruit of the genus Malus ... a fruit of the genus Pyrus or a substance derived therefrom ...".

(15) "... the invention described in claims 5 and 7 has" described on page 32, lines 15 to 16 is deleted, and "... the effect that a mixture of a mature fruit of the genus Malus domestica (apple) with an immature fruit thereof or a fruit of crabapple (Malus domestica) has a higher anitumor effect

or a higher immunopotentiating effect, ..." described on the same page, lines 16 to 19 is amended to "... the effect that a mixture of a mature fruit of the genus *Malus domestica* (apple) with an immature fruit thereof or a fruit of crabapple (*Malus pumilia*) has a higher immunopotentiating effect,...".

(16) "A drug such as an anticancer agent or an immunopotentiator, comprising ..." described in claim 1 on page 34 is amended to "A drug such as an immunopotentiator, comprising ...".

(17) "The drug such as an anticancer agent or an immunopotentiator as described ..." described in claim 2 on page 34 is amended to "The drug such as an immunopotentiator as described ...".

(18) "The drug such as an anticancer agent or an immunopotentiator as described ..." described in claim 3 on page 34 is amended to "The drug such as an immunopotentiator as described ...".

(19) "The drug such as an anticancer agent or an immunopotentiator as described ..." described in claim 4 on page 34 is amended to "The drug such as an immunopotentiator as described ...".

(20) "A health food for inhibiting and preventing cancer and for enhancing immune system ..." described in claim 6 on page 35 is amended to "A health food for enhancing

immune system ...".

6. List of Attached Documents

- (1) Specification: pages 1-2, 4-10, 27, 30-32
- (2) Claims: pages 34-35



## DESCRIPTION

### DRUGS AND HEALTH FOODS INCLUDING IMMUNOPOTENTIATORS

#### Technical Field

5       The present invention relates to drugs and health foods including immunopotentiators for inhibiting and preventing cancer and for enhancing immune system, comprising a fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), a fruit of the genus *Pyrus* such as  
10 a pear, or a substance derived therefrom as a raw material.

Specifically, the present invention relates to drugs and health foods including immunopotentiators for enhancing immune system, comprising as a raw material the above-mentioned fruit of the genus *Malus*, the fruit of the genus  
15 *Pyrus*, a substance derived therefrom as it is or as processed to juice and the like, an extract obtained by extracting the above-mentioned fruit of the genus *Malus* or the fruit of the genus *Pyrus* with water or an organic solvent such as alcohol, or a substance derived from the  
20 extract. The present invention further relates to drugs and health foods including immunopotentiators

for enhancing immune system, comprising a mature fruit and/or an immature fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), or a fruit of the genus *Pyrus* as a raw material.

## 5 Background Art

Conventionally, it has been known that an extract of *Cordyceps sinensis*, when administered as a drug such as an immunopotentiator and a health food, has the effects of enhancing the innate immune system such as natural killer  
10 cells, macrophages and the like, in addition to suppressing the growth of existing cancer cells, and when taken regularly, it further has the effect of preventing the onset of cancer, that is, the cancer preventing effect (for example, see Patent Document 1).

15 In addition, it has also been known that as a health food containing apple juice, which is prepared by mixing apple juice and a proantocyanidine-containing substance, powdering the mixed solution to prepare a powdered composition to subsequently obtain a health food containing  
20 the powdered composition of apple juice as an active ingredient, has anti-oxidation activity, anti-mutagenic activity, anti-ulcer activity, deodorization effect for excrement and preventing effect for diabetic complications, due to the presence of proantocyanidine, in addition to the  
25 effects of the natural ingredients derived from the fruit

been known to contain a large amount of potassium which has a diuretic action and fructose (glucose) which is a source of energy.

However, medical or pharmaceutical studies on the pharmacological effects of the apple have been rarely carried out, but the similar studies on health food containing apple vinegar as a supplementary ingredient have been proposed. However, studies on the immunopotentiating action of the apple have not been conducted.

In other words, a specific study related to the pharmacological effects of apple itself has not been carried out, and the mechanism of the pharmacological effects has not yet been clarified, and further studies on apple as an immunopotentiator, or a cancer-preventing agent have not been tried at all.

Currently, the leading cause of death in Japan is cancer, and surgical operation, radioactive treatment, chemotherapy using an anticancer agent, etc. are commonly carried out as treatments for cancer. However, cancer for which anticancer agents are effective is limited, and

further the side effects from the anticancer agents including death are becoming socially important problems.

On the other hand, humans and animals have inherent immune system which protects the body from pathogenic  
5 microorganisms. Since cancer cells are self-derived foreign substances generated in the body, in the case where the immunity is high, the cancer cells are typically removed by natural killer cells, macrophages and the like.

However, if the immunity is lowered due to aging or  
10 accumulated stress, cancer cells tend to proliferate.

An object of the invention described in claims 1 to 3 and 6 in the present inventions is to enhance the innate immune system such as natural killer cells, macrophages and the like, or the activity of lymphocytes, and thus obtain a  
15 higher immunopotentiating effect by using both a mature fruit and an immature fruit or only a immature fruit of a fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), or a fruit of the genus *Pyrus*.

An object of the invention described in claim 4 is to  
20 enable suitable administration depending on the situation, in addition to the object of the invention described in claims 1 to 3.

## Disclosure of Invention

The present inventors have found that a fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple  
5 (*Malus pumila*), a fruit of the genus *Pyrus*, or a substance derived therefrom, has the higher immunopotentiating effect by initially carrying out various animal tests, and then on humans, to thus complete the present inventions.

To achieve the above-mentioned objects, the invention  
10 described in claim 1 in the present inventions is directed to a drug such as an immunopotentiator, which is characterized by comprising both a mature fruit and an immature fruit, or only an immature fruit of a fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple  
15 (*Malus pumila*), a fruit of the genus *Pyrus*, or a substance derived therefrom as an active ingredient.

The invention described in claim 2 is characterized by the constitution of using an aqueous extract of both mature fruit and an immature fruit, or an immature fruit of the  
20 fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), the fruit of the genus *Pyrus*, or a substance derived from the aqueous extract, in addition to the constitution of the invention described in claim 1.

The invention described in claim 3 is characterized by  
25 the constitution of using an extract both mature fruit and

an immature fruit, or an immature fruit of the fruit of the  
genus *Malus* such as apple (*Malus domestica*) and crabapple

(*Malus pumila*), the fruit of the genus *Pyrus* obtained by extraction with an organic solvent such as alcohol, or a substance derived from the extract, in addition to the constitution of the invention described in claim 1.

5       The organic solvent such as alcohol herein includes, for example, acetone, ether, ethyl acetate, chloroform, and methylene chloride.

It has been revealed that the effects of invention described in claims 1 to 3 obtained from such constitutions,  
10       consist in that both the mature fruit and the immature fruit, or only immature fruit of a fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), a fruit of the genus *Pyrus*, a substance therefrom, an extract obtained by extracting the fruit with water or an  
15       organic solvent such as alcohol, or a substance derived from the extract, when administered, enhances the immune system such as natural killer cells, macrophages and the like to control cancer-attacked cells, as well as to prevent carcinogenesis of cells not yet attacked by cancer, and when  
20       taken regularly, obtain higher immunopotentiating effect and thus has the effect of preventing the onset of cancer, that is, the cancer preventing effect.

The invention described in claim 4 is characterized by the constitution of administering as a raw material an  
25       extract of both the mature fruit and the immature fruit, or

only immature fruit of the fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), the fruit of the genus *Pyrus*, or a substance derived from the extract orally,



percutaneously, parenterally, or via other routes, in addition to the constitution of the invention described in claim 1, 2 or 3.

5 The effect of invention described in claim 4 obtained from such constitution in addition to the effects of the invention described in claim 1, 2 or 3, consists in that it enables percutaneous administration, parenteral administration and administration via other routes, although oral administration is a general administration route.

10 The invention described in claim 6 is a health food for enhancing immune system, comprising the mature fruit and the immature fruit, or only immature fruit of a fruit of the genus Malus such as apple

(Malus domestica) and crabapple (Malus pumila), a fruit of the genus Pyrus, or a substance derived therefrom as a raw material.

It has been proved that the effect of invention  
5 described in claim 6 obtained from such constitution  
consists in that a fruit of the genus Malus such as apple  
(Malus domestica) and crabapple (Malus pumila), a fruit of  
the genus Pyrus, or a substance derived therefrom, when  
administered, enhances the immune system such as natural  
10 killer cells, macrophages and the like to control cancer-  
attacked cells, as well as to prevent carcinogenesis of  
cells not yet attacked by cancer, and when taken regularly,  
has the effect of preventing the onset of cancer, that is,  
the cancer preventing effect.

#### Brief Description of the Drawings

Fig. 1 is a graph showing a tumor growth curve (mean value) of mice from each test group after tumor MethA  
5 inoculation.

Fig. 2 is a graph showing the phagocytosis ability of peritoneal macrophages of mice having no tumor inoculation and in mice in which tumor has been cured after tumor inoculation.

10 Fig. 3 is a graph showing the change of the activity of natural killer cells in a subject orally administered with apple in a form of juice every day for 5 weeks.

#### Best Mode for Carrying out the Invention

In the following, Example of the present invention will  
15 be explained with reference to Drawings.

This Example shows the case where fruit juice of apple *Malus domestica* was orally administered as a fruit of the genus *Malus*.

40 female 4-week-old BALB/c mice (CLEA Japan, Inc.)  
20 were divided into four test groups (10 mice/group), i.e., a test group administered with fruit juice of apple *Malus domestica*, a group administered with the extract of the fruiting body of *Cordyceps sinensis*, a test group administered with a mixture of the extract of the insect  
25 body part of *Cordyceps sinensis*, and a control group

weight, as compared to apple *Malus domestica*, were investigated in the same manner as in the above-described test. The fruit was made into a juice with the fruit skin included and the fruit core removed by a juicer and taken by mice. Further, the antitumor effect was investigated when crabapple (*Malus pumila*) was made into a juice by a juicer with the fruit skin included, and mixed with a juice of a mature fruit of the genus *Malus domestica*. This was carried out for the purpose of developing a functional food or a drug which elevates the antitumor effect and the immunopotentiating effect by enhancing the frequencies of pectin and polyphenol by mixing the pectin which is contained in a large amount in the juice of a mature fruit of the genus *Malus domestica*, with the polyphenol which is contained in a large amount in a fruit of the genus *Malus pumila* (crabapple) or a fruit of the genus *Pyrus*.

As the results of the test for the mice, it has been observed that a juice of a mature fruit of the genus *Malus domestica* which is mixed with a juice of an immature fruit thereof, a fruit juice of crabapple (*Malus pumila*), or with the genus *Pyrus*, when administered, has a higher antitumor effect or immunopotentiating effect, as compared to a juice of a mature fruit of the genus *Malus domestica* alone. Further, it has been proven that such mechanism activates the "innate immune system" such as natural killer the fruit

of the genus *Malus* obtained by extraction with an organic solvent such as alcohol, or a substance derived from the extract, was used and administered percutaneously, parenterally or via the routes other than an oral route.

5        In addition, the same effects as in the above Examples were obtained by the tests wherein, the above-described fruit such as an apple or a pear was administered in a mixture with, for example, a fruit of the genus *Prunus* such as an apricot, a plum, a peach or a mountain cherry, a fruit  
10 of the genus *Chaenomeles* such as a Flowering quince, a fruit of the genus *Cydonia* such as a marmelo, a fruit of the genus *Diospyros* such as a persimmon, a fruit of the genus *Morus* such as a mulberry, a fruit of the genus *Rosa* such as a Japanese rose, a fruit of the genus *Poncirus* such as a  
15 trifoliolate orange, or a fruit of the genus *Eriobotrya* such as a loquat, or wherein, for example, a fruit of the genus *Prunus* such as an apricot, a plum, a yellow peach or a mountain cherry, a fruit of the genus *Chaenomeles* such as a Flowering quince, a fruit of the genus *Cydonia* such as a  
20 marmelo, a fruit of the genus *Diospyros* such as a persimmon, a fruit of the genus *Morus* such as a mulberry, a fruit of the genus *Rosa* such as a Japanese rose, a fruit of the genus *Poncirus* such as a trifoliolate orange, or a fruit of the genus *Eriobotrya* such as a loquat, was administered alone.

As described above, it has been observed that the invention described in claims 1 to 3 or 6 in the present inventions has the effects that both a mature fruit and an immature fruit, or only an immature fruit of a fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), a fruit of the genus *Pyrus*, a substance derived therefrom, an extract by extracting the fruit with water or an organic solvent such as alcohol, or a substance derived from the extract, when administered, enhances the innate immune system such as natural killer cells, macrophages and the like to control cancer-attacked cells, as well as to prevent carcinogenesis of cells not yet attacked by cancer, and when taken regularly, has the effect of preventing the onset of cancer, that is, the cancer preventing effect.

In other words, administration of a fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), a fruit of the genus *Pyrus*, or a substance derived therefrom could enhance the innate immune system such as natural killer cells, macrophages and the like, and the activity of lymphocytes, inhibit the onset or growth of cancer cells, and prevent or suppress cancer.

Further, it has been proven that it could suppress or control cancer more effectively, as compared to the extract of *Cordyceps sinensis* which has conventionally been

suggested as an immunopotentiator.

Further, it has been found that by administering a

fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), a fruit of the genus *Pyrus*, or a substance derived therefrom to cancer patients, QOL of the cancer patients is improved, and remarkable life-prolonging effect is obtained.

Accordingly, it can be widely administered since it is not expensive and is more easily available, as compared to the conventional extract of *Cordyceps sinensis*.

The invention described in claim 4 has the effect of enabling suitable administration depending on the situation percutaneously, parenterally or via other routes, although oral administration is a general administration route, in addition to the effects of the invention described in claims 1 to 3.

It has been proven that the effect that a mixture of a mature fruit of the genus *Malus domestica* (apple) with an immature fruit thereof or a fruit of crabapple (*Malus pumila*) has a higher immunopotentiating effect, as compared to a mature fruit of the genus *Malus domestica* alone, in addition to the effects of the invention described in claims 1 to 4 or 6.

Further, in the case of using an immature fruit, an immature fruit which has conventionally been thinned out and discarded can be harvested and utilized as a juice. In



## CLAIMS

1. (Amended) A drug such as an immunopotentiator, comprising both a mature fruit and an immature fruit, or an immature fruit of a fruit of the genus *Malus* such as apple  
5 (*Malus domestica*) and crabapple (*Malus pumila*), a fruit of the genus *Pyrus*, or a substance derived therefrom as an active ingredient.

2. (Amended) The drug such as an immunopotentiator as described in claim 1, comprising an aqueous extract of both  
10 the mature fruit and the immature fruit, or the immature fruit of the fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), the fruit of the genus *Pyrus*, or a substance derived from the aqueous extract.

15 3. (Amended) The drug such as an immunopotentiator as described in claim 1, comprising an extract of both the mature fruit and the immature fruit, or the immature fruit of the fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), the fruit of the  
20 genus *Pyrus* obtained by extraction with an organic solvent such as alcohol, or a substance derived from the extract.

4. (Amended) The drug such as an immunopotentiator as described in claim 1, 2 or 3, administered orally, percutaneously, parenterally or via other routes, which  
25 comprises the extract of both the mature fruit and the

immature fruit, or the immature fruit of the fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), the fruit of the genus *Pyrus*, or a substance derived from the extract as a raw material.

5      5. (Deleted at the stage of amendment under Art. 19)

6. (Amended) A health food for enhancing immune system, comprising both a mature fruit and an immature fruit, or an immature fruit of a fruit of the genus *Malus* such as apple (*Malus domestica*) and crabapple (*Malus pumila*), a fruit of  
10 the genus *Pyrus*, or a substance derived therefrom as a raw material.

7. (Deleted at the stage of amendment under Art. 19)